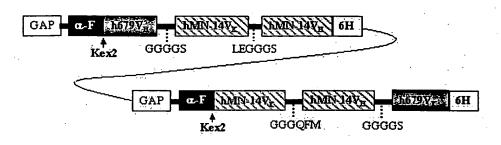
A.



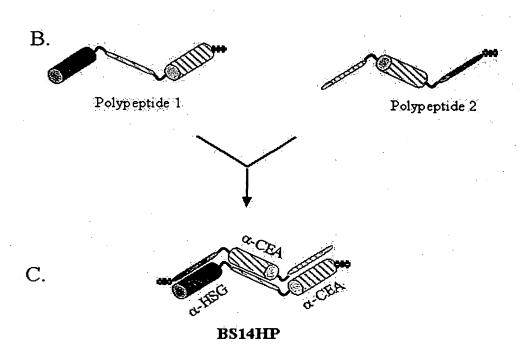


Figure 1

#### FIGURE 1D

1) Amino acid sequence of Polypeptide 1. EAEAEFM-h679VH-GGGGS-hMN-14VK-LEGGGS-hMN-14VH-VD6His.

<u>EAEAEFM</u>EVQ LVESGGDLVK PGGSLKLSCA ASGFTFS<u>IYT MS</u>WLRQTPGK CDR1h679VH

GLEWVA**TLSG DGDDIYYP**DS VKGRFTISRD NAKNSLYLQM NSLRAEDTAL CDR2h679VH

YYCAR<u>VRLGD WDFDV</u>WGQGT TVSVSS<u>GGGG S</u>DIQLTQSPS SLSASVGDRV CDR3h679VH linker

TITC<u>KASQDV GTSVA</u>WYQQK PGKAPKLLIY <u>WTSTRHT</u>GVP SRFSGSGSGT CDR1hMN14VK CDR2hMN14VK

DFTFTISSLQ PEDIATYYC**Q QYSLYRS**FGQ GTKVEIKR<u>LE GGGS</u>EVQLVE CDR3hMN14VK linker

SGGGVVQPGR SLRLSCSASG FDFT<u>TYWMS</u>W VRQAPGKGLE WIG<u>EIHPDSS</u> CDR1hMN14VH CDR2hMN14VH

TINYAPSLKD RFTISRDNAK NTLFLQMDSL RPEDTGVYFC ASLYFGFPWF CDR2hMN14VH CDR3hMN14VH

AYWGQGTPVTVS<u>VDHHHHHH</u> CDR3hMN14VH 6His

Nucleic acid sequence (cDNA) of BS14HP polypeptide 1

GAGGCTGAAG CTGAATTCAT GGAAGTGCAG CTGGTGGAGT CAGGGGGAGA CTTAGTGAAG CCTGGAGGGT CCCTGAAACT CTCCTGTGCA GCCTCTGGAT TCACTTCAG TATTTACACC ATGTCTTGGC TTCGCCAGAC TCCGGGAAAG GGGCTGGAGT GGGTCGCAAC CCTGAGTGGT GATGGTGATG ACATCTACTA TCCAGACAGT GTGAAGGGTC GATTCACCAT CTCCAGAGAC AATGCCAAGA ACAGCCTATA TCTGCAGATG AACAGTCTAA GGGCTGAGGA CACGGCCTTG TATTACTGTG CAAGGGTGCG ACTTGGGGAC TGGGACTTCG ATGTCTGGGG CCAAGGACC ACGGTCTCCG TCTCCTCAGG AGGTGGCGGA TCCGACATCC AGCTGACCCA GAGCCCAAGC AGCCTGAGCG CCAGCGTGGG TGACAGAGTG ACCATCACCT GTAAGGCCAG TCAGGATGTG GGTACTTCTG TAGCTTGGTA CCAGCAGAAG CCAGGTAAGG CTCCAAAGCT GCTGATCTAC TGGACATCCA CCCGGCACAC TGGTGTGCCA AGCAGATTCA GCGGTAGCGG TAGCGGTACC GACTTCACCT TCACCATCAG CAGCCTCCAG CCAGAGGACA TCGCCACCTA CTACTGCCAG CAATATAGCC TCTATCGGTC GTTCGGCCAA GGGACCAAGG TGGAAATCAA ACGTCTCGAG GGCGGAGGTA GCGAGGTCCA ACTGGTGGAG AGCGGTGGAG GTGTTGTGCA ACCTGGCCGG TCCCTGCGCC TGTCCTGCTC CGCATCTGGC TTCGATTTCA CCACATATTG GATGAGTTGG GTGAGACAGG CACCTGGAAA AGGTCTTGAG TGGATTGGAG AAATTCATCC AGATAGCAGT

ACGATTAACT ATGCGCCGTC TCTAAAGGAT AGATTTACAA TATCGCGAGA CAACGCCAAG AACACATTGT TCCTGCAAAT GGACAGCCTG AGACCCGAAG ACACCGGGGT CTATTTTGT GCAAGCCTTT ACTTCGGCTT CCCCTGGTTT GCTTATTGGG GCCAAGGGAC CCCGGTCACC GTCTCCGTCG ACCATCATCA TCATCATCAT

#### FIGURE 1E

3) Amino acid sequence of polypeptide 2. EAEAEF-hMN-14VK-GGGQFM-hMN-14VH-GGGGS-h679VK-LD6His.

#### <u>EAEAEF</u>DIQL TQSPSSLSAS VGDRVTITC<u>K ASQDVGTSVA</u> WYQQKPGKAP CDR1hMN14VK

KLLIY<u>WTSTR HT</u>GVPSRFSG SGSGTDFTFT ISSLQPEDIA TYYC<u>OQYSLY</u> CDR2hMN14VK CDR3hMN14VK

RSFGQGTKVE IKRGGGOFME VQLVESGGGV VQPGRSLRLS CSASGFDFTT CDR3hMN14VK linker CDR1hMN14VH

YWMSWVRQAP GKGLEWIGEI HPDSSTINYA PSLKDRFTIS RDNAKNTLFL CDR1hMN14VH CDR2hMN14VH

QMDSLRPEDT GVYFCAS<u>LYF GFPWFAY</u>WGQ GTPVTVS<u>GGG GS</u>DIVMTQSP CDR3hMN14VH linker

SSLAVSPGER VTLTC<u>KSSQS LFNSRTRKNY LG</u>WYQQKPGQ SPKLLIY<u>WAST</u> CDR1h679VK CDR2h679VK

RESGVPDRFS GSGSGTDFTL TINSLQAEDV AVYYCTQVYY LCTFGAGTKLE CDR2h679VK CDR3h679VK

LKRLDHHHHH H 6His

#### Nucleic acid sequence (cDNA) of BS14HP polypeptide 2

GAGGCTGAAG CTGAATTCGA CATCCAGCTG ACCCAGAGCC CAAGCAGCCT
GAGCGCCAGC GTGGGTGACA GAGTGACCAT CACCTGTAAG GCCAGTCAGG
ATGTGGGTAC TTCTGTAGCT TGGTACCAGC AGAAGCCAGG TAAGGCTCCA
AAGCTGCTGA TCTACTGGAC ATCCACCCGG CACACTGGTG TGCCAAGCAG
ATTCAGCGGT AGCGGTAGCG GTACCGACTT CACCTTCACC ATCAGCAGCC
TCCAGCCAGA GGACATCGCC ACCTACTACT GCCAGCAATA TAGCCTCTAT
CGGTCGTTCG GCCAAGGGAC CAAGGTGGAA ATCAAACGTG GAGGTGGCCA
ATTCATGGAG GTCCAACTGG TGGAGAGCGG TGGAGGTGTT GTGCAACCTG
GCCGGTCCCT GCGCCTGTCC TGCTCCGCAT CTGGCTTCGA TTTCACCACA
TATTGGATGA GTTGGGTGAG ACAGGCACCT GGAAAAAGGTC TTGAGTGGAT
TGGAGAAATT CATCCAGATA GCAGTACGAT TAACTATGCG CCGTCTCTAA
AGGATAGATT TACAATATCG CGAGACAACG CCAAGAACAC ATTGTTCCTG
CAAATGGACA GCCTGAGACC CGAAGACACC GGGGTCTATT TTTGTGCAAG
CCTTTTACTTC GGCTTCCCT GGTTTGCTTA TTGGGGCCAA GCGACCCCGG
TCACCGTCTC CGGAGGCGGT GGATCCGACA TTGTGATGAC ACAATCTCCA

maamaaamaa	OMOMOMON OO	~~~~~~~		~~~~~
TCCTCCCTGG	CTGTGTCACC	CGGGGAGAGG	GTCACTCTGA	CCTGCAAATC
CAGTCAGAGT	CTGTTCAACA	GTAGAACCCG	AAAGAACTAC	TTGGGTTGGT
ACCAGCAGAA	ACCAGGGCAG	TCTCCTAAAC	TTCTGATCTA	CTGGGCATCT
ACTCGGGAAT	CTGGGGTCCC	TGATCGCTTC	TCAGGCAGTG	GATCCGGAAC
AGATTTCACT	CTCACCATCA	ACAGTCTGCA	GGCTGAAGAC	GTGGCAGTTT
ATTACTGCAC	TCAAGTTTAT	TATCTGTGCA	CGTTCGGTGC	TGGGACCAAG
CTGGAGCTGA	AACGGCTCGA	ССАТСАТСАТ	САТСАТСАТ	

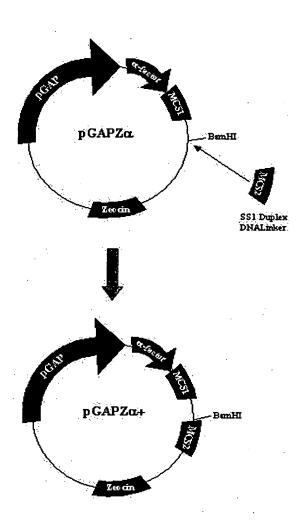


Figure 2

## BIAcore analysis of BS14HP

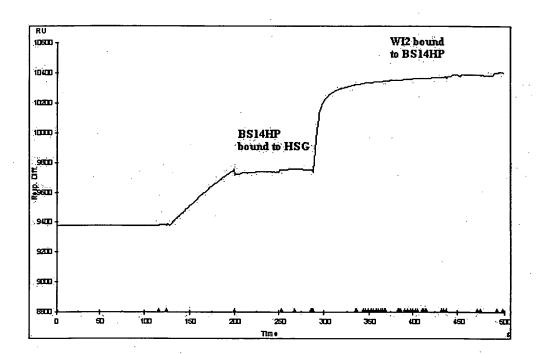
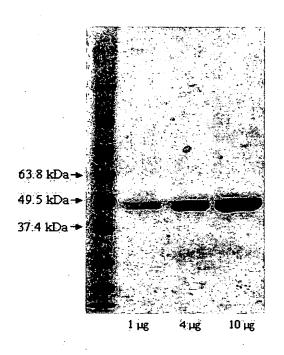


Figure 3

### SDS-PAGE Analysis of BS14HP B/N 111802

# BEST AVAILABLE COPY



Calculated MW of polypeptides 40.06 kDa & 40.61 kDa

Figure 4

Size exclusion HPLC analysis

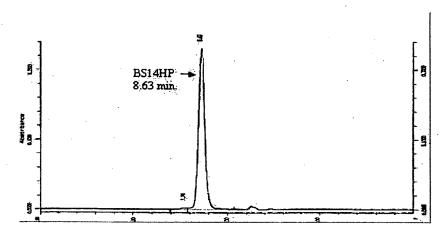


Figure 5

### Competitive ELISA assay for CEA binding

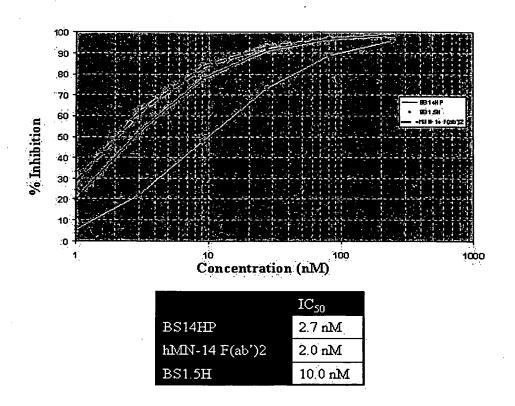


Figure 6

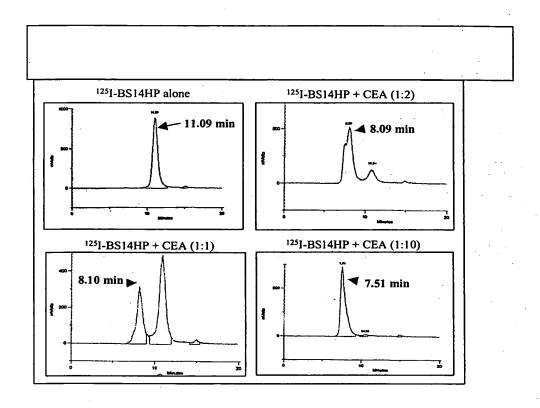


Figure 6B SE-HPLC Analysis of BS14HP immunoreactivity with CEA

# Tumor retention and blood clearance of $^{125}IBS14HP$

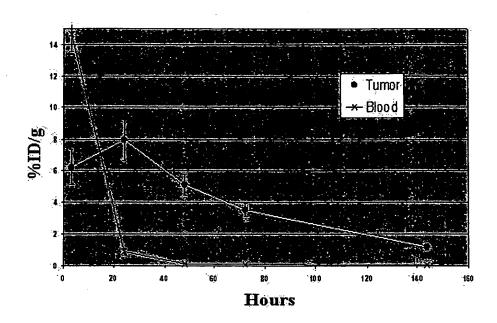
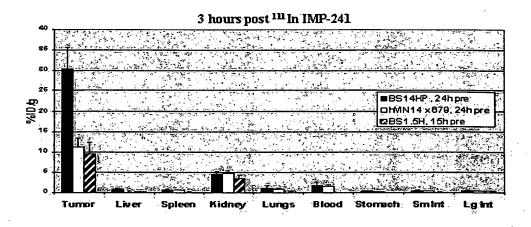


Figure 7

A. Biodistribution of <sup>111</sup>In-IMP241in mice pretargeted with BS14HP, BS1.5H or hMN14 x 679 Fab' x Fab'



B. Tumor/non-tumor ratios of <sup>111</sup>In-IMP241 in mice pretargeted with BS14HP, BS1.5H or hMN-14 x 679 Fab' x Fab'

	BS14HP	hMN-14 x 679	B\$1.5H
Liver	36.19 (±18.8)	22.20 (±6.3)	120.00 (±36.0)
Spleen	57.39 (±46.0)	27.80 (±5.9)	181.00 (±58.0)
Kidney	6.70 (±0.7)	2.50 (±0.5)	2.98 (1.1±)
Lungs	29.94 (±15.6)	14.10 (±2.8)	48.60 (±19.3)
Blood	20.32 (±34.7)	8.10 (±2.1)	284.00 (±50.6)
Stomach	123.12 (±242.0)	103.00 (±15.2)	530.00 (±291.7)
Sm. Intestine	78.95 (±55.0)	53.40(±14.4)	235.00 (±138.7)
Lg. Intestine	80.94 (±26.7)	37.40 (±9.2)	61.20 (±33.2)

Figure 8

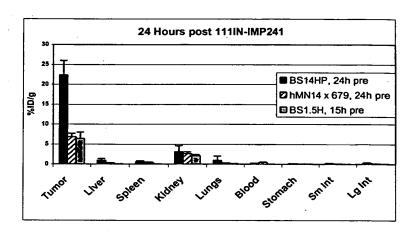


Figure 8C

### **IMP 281**

Figure 9A

Figure 9B

Figure 9C

### Functional features of the SV3 shuttle vector.

HindIII—Xhol—Xbal—Leader peptide—NcoI—SalI—6His—Stop—Stop—BglII—Eagl—EcoRI

### Figure 10 A

### Features of the ORF/Polypeptide 1, and ORF/Polypeptide 2

ORF1/Polypeptide 1
Ldr Pep—h679V<sub>H</sub>—GGGGS—hMN-14V<sub>K</sub>—LEGGGS—hMN-14V<sub>H</sub>—HHHHHHH

ORF2/Polypeptide 2
Ldr Pep—hMN-14V<sub>K</sub>—GGGQFM—hMN-14V<sub>H</sub>—GGGGS— h679V<sub>K</sub>—HHHHHHH

Figure 10B

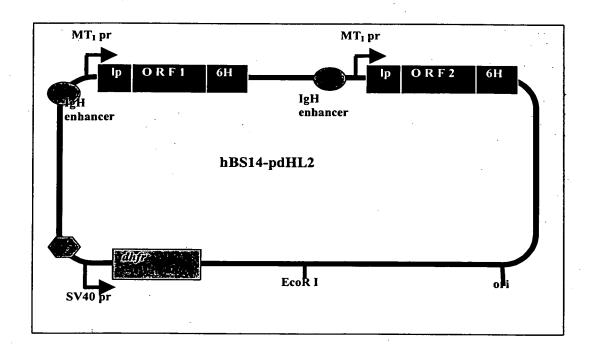


Figure 11. Schematic representation of hBS14-pDHL2 expression vector.

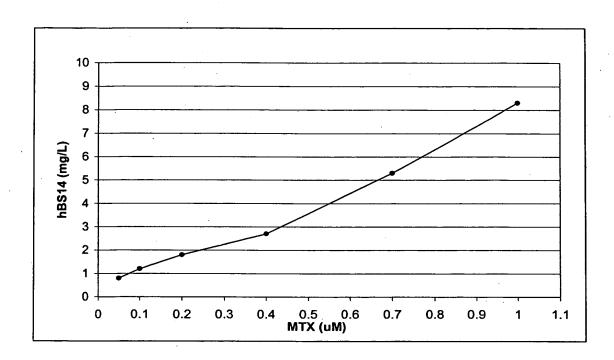


Figure 12. MTX amplification of hBS14 SP2/0 clone 1H6

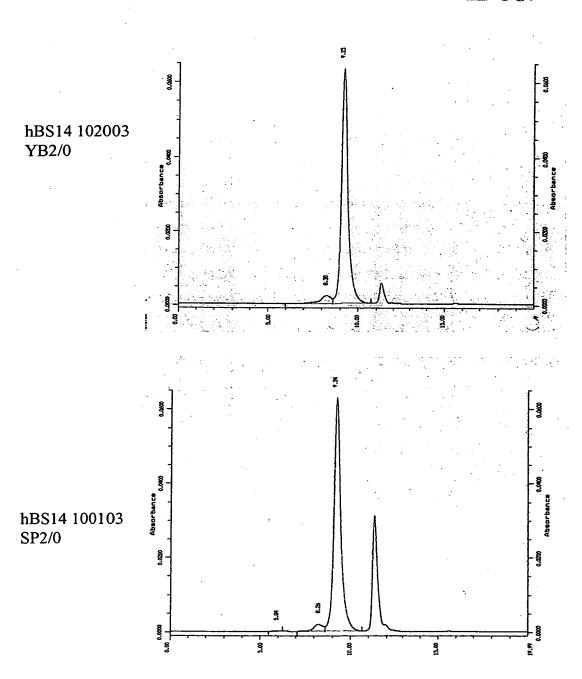


Figure 13. SE-HPLC analysis of purified hBS14

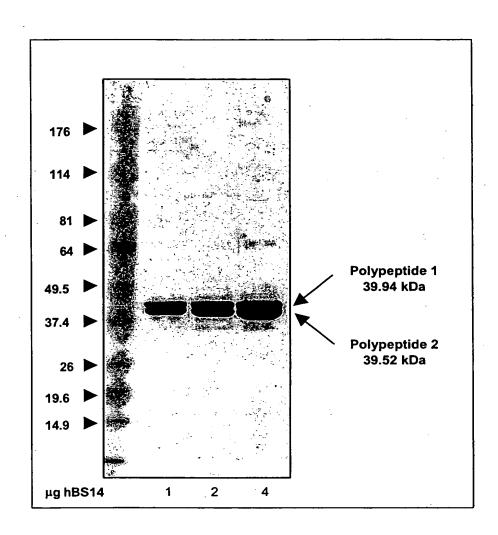


Figure 14. SDS-PAGE analysis of purified hBS14

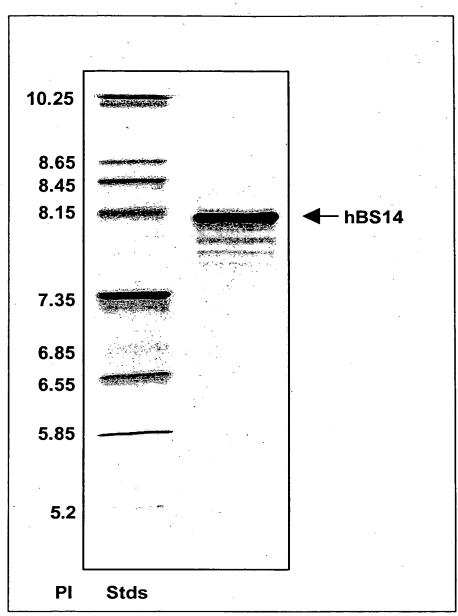


Figure 15. IEF analysis of purified hBS14

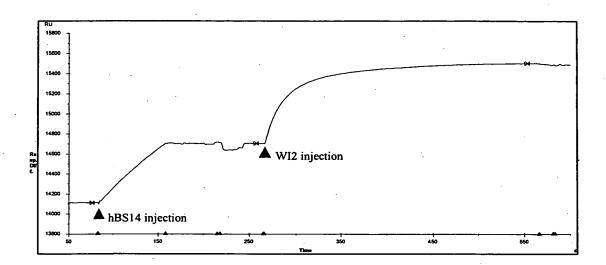


Figure 16. BIAcore analysis of hBS14

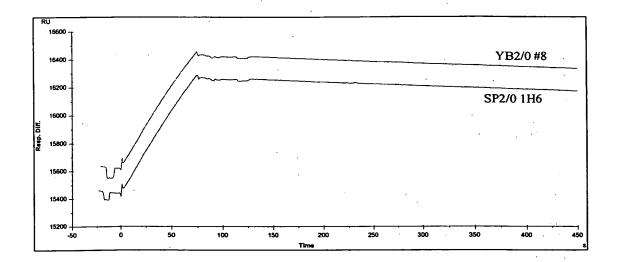


Figure 17. BIAcore analysis of HSG binding of hBS14 produced in either SP2/0 or YB2/0 cells

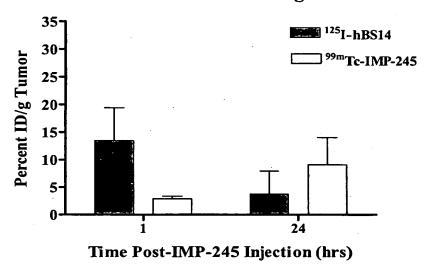
Figure 18

IMP 291 Ac-Lys(HSG-iAsp-)-Cys-NH<sub>2</sub> MH<sup>+</sup> 656

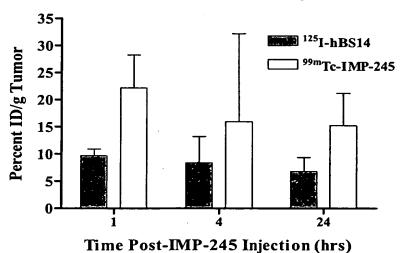
Figure 19

IMP 245 DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-Lys(Tscg-Cys-)-NH<sub>2</sub> MH<sup>+</sup> 1832

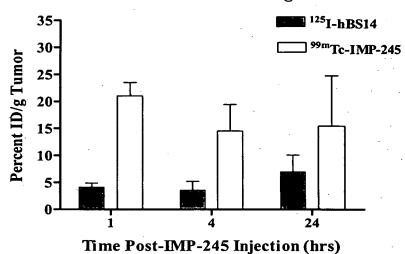
Pre-Targeting of <sup>99m</sup>Tc-IMP-245 by hBS14 (4 hr) in GW-39 Tumor-Bearing Mice



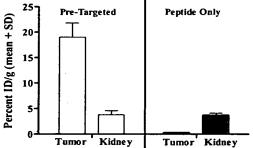
Pre-Targeting of <sup>99m</sup>Tc-IMP-245 by hBS14 (24 hr) in GW-39 Tumor-Bearing Mice



Pre-Targeting of <sup>99m</sup>Tc-IMP-245 by hBS14 (48 hr) in GW-39 Tumor-Bearing Mice



<sup>99m</sup>Tc-IMP-245 Uptake in Imaged Mice at 24 hr Post-Injection



Percent I	Percent ID/g and Tumor Non-Tumor Ratios of 99mTc-IMP-245 at 1 hr Post-Injection.	or Non-Tumo	r Ratios of <sup>99m</sup>	Tc-IMP-245	at 1 hr Post-In	njection.
	4 hrs	4 hrs hBS14	24 hrs hBS14	BS14	48 hr	48 hr hBS14
	Clearance	ance	Clearance	ance	Clean	Clearance
		T:NT Ratio		T:NT Ratio		T:NT Ratio
Tissue	%ID/g ± (SD)	± (SD)	$\%ID/g \pm (SD)$	± (SD)	$\%ID/g \pm (SD)$	±(SD)
GW-39	2.9 ± 0.5	2.9 ± 0.51	22.2 ± 6.1.		(三,21.0) 主2.5	
Liver	$8.3 \pm 0.6$	$0.4 \pm 0.07$	$1.5 \pm 0.8$	$17.5 \pm 6.5$	$1.1 \pm 0.2$	$19.7 \pm 3.6$
Spleen	7.5 ± 1.9	$0.4 \pm 0.08$	$0.9 \pm 0.3$	27.7 ± 9.4	$0.5 \pm 0.1$	$41.3 \pm 9.7$
Kidney	13.4 ± 11.1		0.2/± 0.03	4.11王07	8:0 ∓ 6:2	2.7±0.5
Lungs	$10.7 \pm 3.1$	$0.3 \pm 0.10$	$1.7 \pm 0.7$	$14.1 \pm 4.8$	$1.0 \pm 0.4$	$23.0 \pm 6.8$
Blood	36.9 ±16.4	○0.1 ± 0:01 ※	7.11±18.7	0.11年01017   2.4.7.41年8734   23代57年37年37	1.6 ± 0.3	13.6 ± 2.3
Stomach	$1.3 \pm 0.4$	$2.4 \pm 0.67$	$4.0 \pm 7.6$	$29.4 \pm 18.8$	$3.5 \pm 0.9$	$6.4 \pm 2.0$
Small Int.	$3.7 \pm 0.3$	$0.8 \pm 0.07$	3.9 ± 5.3	$11.8 \pm 6.4$	$2.4 \pm 0.5$	$8.8 \pm 1.5$
Large Int.	$2.2 \pm 1.7$	$1.8 \pm 0.98$	$0.4 \pm 0.2$	$69.4 \pm 31.2$	$0.4 \pm 0.1$	$56.0 \pm 11.5$
Muscle	$1.2 \pm 0.2$	$2.4 \pm 0.32$	$4.8 \pm 6.4$	$19.1 \pm 26.3$	$4.1 \pm 5.0$	$18.4 \pm 25.6$
Tumor	1		,		ļ	
Weight (grams)	$0.309 \pm 0.139$	: 0.139	0.309	$0.309 \pm 0.136$	0.972	$0.972 \pm 0.640$
(Et a 1113)						

Figure 23

